Siro KURITA*: **Chromosome numbers of some Japanese ferns (4)****

栗田子郎*: 日本産シダ類数種の染色体数 (4)**

This is an extention of a series of cytotaxonomical studies on Japanese ferns (Kurita, 1960, 1961, 1962, 1963). Since the author published the first paper which treated the number of gametic chromosomes in 24 taxa, he has investigated chromosomes in about 100 species in total. Based on his more recent cytological findings and on morphological information so far obtained from many sources, the author will discuss in this paper systematic positions of 18 more Japanese species (10 genera)and a formosan fern, counting chromosomes in each of them.

The names of the examined species and their localities are shown in the text. For cytology, the material was fixed with 1 : 3 acetic acid-alcohol, squashed in acetocarmine, and was made permanent for observation and microphotography following the common method (cf. Manton, 1950).

Observations and Discussions

1) *Adiantum capillus-veneris* L.

This fern was collected at Hamaoka, Shizuoka Prefecture. As shown in Fig. a, 30 bivalent chromosomes were counted in many spore mother celles in the first meiotic metaphase and diakinesis. Each sporangium has 16 spore mother cells which give rise to 64 tetrahedral spores. This gametic chromosome number, n=30, has often been reported by many authors (Manton, 1950; Roy and Sinha, 1956, 1958; Mehra and Verma, 1960; Roy and Pandey, 1963; etc.). On the other hand, specimens from, West Africa (Manton, 1959) and N. America (Wagner, 1963) show tetraploidy.

2) *Adiantam edgeworthii* Hook.

Specimens were collected in Ôita, Ôita Prefecture, by Mr. Takaoka. Evidently 30 gametic chromosomes are counted at the first meiotic metaphase and late diakinetic stage (Fig. b). The sporogenesis is normal; 16-celled sporangium yields 64 tetrahedral spores. The same species from eastern Himalaya was

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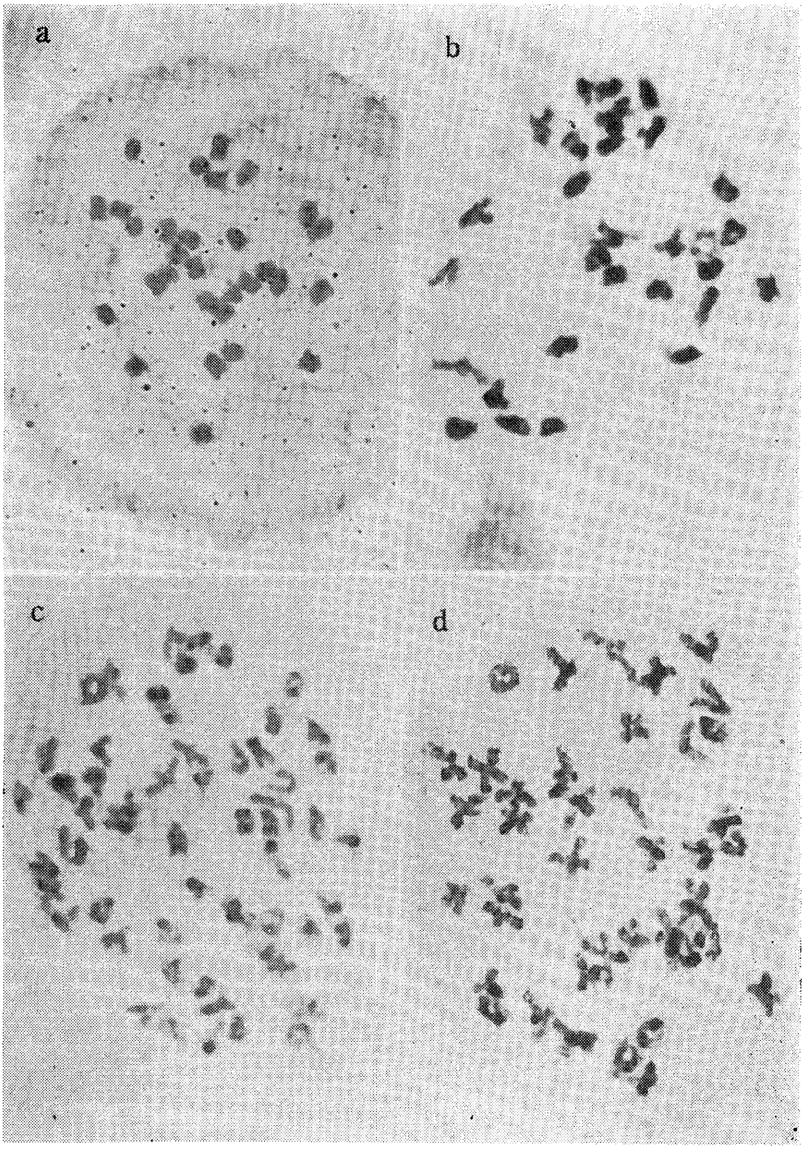

Fig. a. A spore mother cell of *Adiantum capillus-veneris*, showing 30 bivalent chromosomes. × c. 1000. Fig. b. A spore mother cell of *A. edgeworthii*, showing 30 bivalents. × c. 1000. Fig. c. A spore mother cell of *A. monochlamys*, showing 60 bivalents. × c. 1000. Fig. d. A spore mother cell of *Bolbitis subcordata*, showing 41 bivalents. × c. 1000.

studied by Verma (ex Mehra, 1961) who found the haploid number to be 60. The present Japanese species is a diploid, whereas the Himalayan must be considered a tetraploid.

3) *Adiantum monochlamys* Eaton

Specimens were obtained from Ôma, Shizuoka Prefecture. Each spore mother cell shows 60 bivalent chromosomes at late diakinetic stage (Fig. c). Sixteen spore mother cells in each sporangium give rise to 64 tetrahedral spores. Therefore this is a tetraploid species based on x=30.

4) *Athyrium pinetorum* Tagawa

Material was collected at Mt. Togakushi, Nagano Prefecture. Obviously 40 gametic chromosomes are observed at the first meiotic metaphase. Each sporangium contains 16 spore mother cells which give rise to 64 bilateral spores. This species is alpine and is diploid, but in gross morphology it resembles *A. deltoidofrons* which is distributed rather in lowlands showing n=120 (Kurita, 1960: reported as *A. multiudum* Ros.).

5) *Bolbitis subcordata* (Copel.) Ching

The gametic chromosome number of genus *Bolbitis* has been counted by many. The first cytological work on this genus was published by Manton and Sledge (1954) who observed 82 bivalent chromosomes in materials from Malaya and Ceylon. Abraham, Ninan and Mathew (1962) found n=41 in the material from India. Brownlii (ex Fabbri, 1963), on the other hand, counted the gametic chromosome to be 82 in his material from New Zealand. It is certain from these studies that the basic chromosome number is 41 in this genus. In Japan it is found only one species, *B. subcordata*, of which gametic chromosomes are 41 (Fig. d). It is a diploid. There have been arguments about the systematic position of this genus. Holttum (1949) considers it a member of Lomariopsidoideae which includes *Egenolphia* and *Lomariopsis*, and is a derivative of *Microlepia* derived from *Dennstaedtia*,, closely related to Davallioideae and Oleandroideae. Meanwhile, Copeland (1947) places *Bolbitis* in his Aspidiaceae, regarding it as a relative of *Egenolphia*, but he finds no direct affinity of this genus to other dryopteroid genera. Our present knowledge about cytology is not sufficient so far to elucidate the systematic position of this genus.

6) *Camptosorus sibiricus* Rupr.

In the genus *Camptosorus* only two species, *C. rhizophyllus* and *C. sibiricus*, are known. While the former is found in North America, the latter species which is treated here is mainly distributed in eastern Asia, usually on lime stone rocks. Material was collected at Mt. Togakushi, Nagano Prefecture. Many spore mother cells show 36 bivalent chromosomes at the first meiotic division. The sporogenesis is completely normal, namely, 16-celled sporangium yieleds 64 bilateral spores. The same chromosome number was found in *C. rhizophyllus* by Wanger (1953, 1954) who treated this species as a member of *Asplenium*. Britton (1953) counted $n=35$ in *C. rhizophyllus* and considered the genus to be aneuploid, related to *Phyllitis*. Certain hybrid species of *Camptosorus* and *Asplenium* are known to occur readily (Wagner, 1954), and close relation of these genera is doubtless.

7) *Ctenitopsis sinii* (Ching) Ching

The genus *Ctenitopsis* was coined by Ching (1938) who treated it as a member of tectarioid ferns. Copeland (1947) included *Ctenitopsis* in the genus *Ctenitis*, whereas Holttum (1949) considered that almost all the species of *Ctenitopsis* should belong to *Tectaria*. The present species, *C. sinii*, is fairly distinctive among others in this genus; it has some common characters with *Ctenitis* (Tagawa, 1959). The material was obtained from Kagoshima Prefecture. Definitely 41 gametic chromosomes are counted at the first meiotic division (Fig. e). Each sporangium has 16 spore mother cells which give rise to 64 bilateral spores. The basic chromosome number of *Ctenitis* is known to be 41, whereas that of *Tectaria* is always 40. Since it is reasonable to consider *Ctenitopsis* to be more related to *Ctenitis* than to *Tectaria*.

8) *Diplazium doederleinii* (Luerss.) Makino

Material was obtained from the Koishikawa Botanical Gardens, University of Tokyo. Its original locality is Yakushima Island, Kagoshima Prefecture. Each sporangium contains 8 spore mother cells which develop into 32 bilateral spores. Exactly 123 bivalent chromosomes are counted in many spore mother cells at the first meiotic division (Fig. f.). The number of spore mother cells and of spores, 8 and 32 respectively, suggest the apogamous nature of this species. Momose has also confirmed its apogamous nature by the investigation of gametophytes (private communication). Thus the present species is considered an apogamous triploid.

9) *Diplazium pin-faense* Ching

This species is very rare in Japan. Tagawa (1942) studied only one specimen from Amakusa, Kumamoto Prefecture, and identified it with *D. pin-faense* with

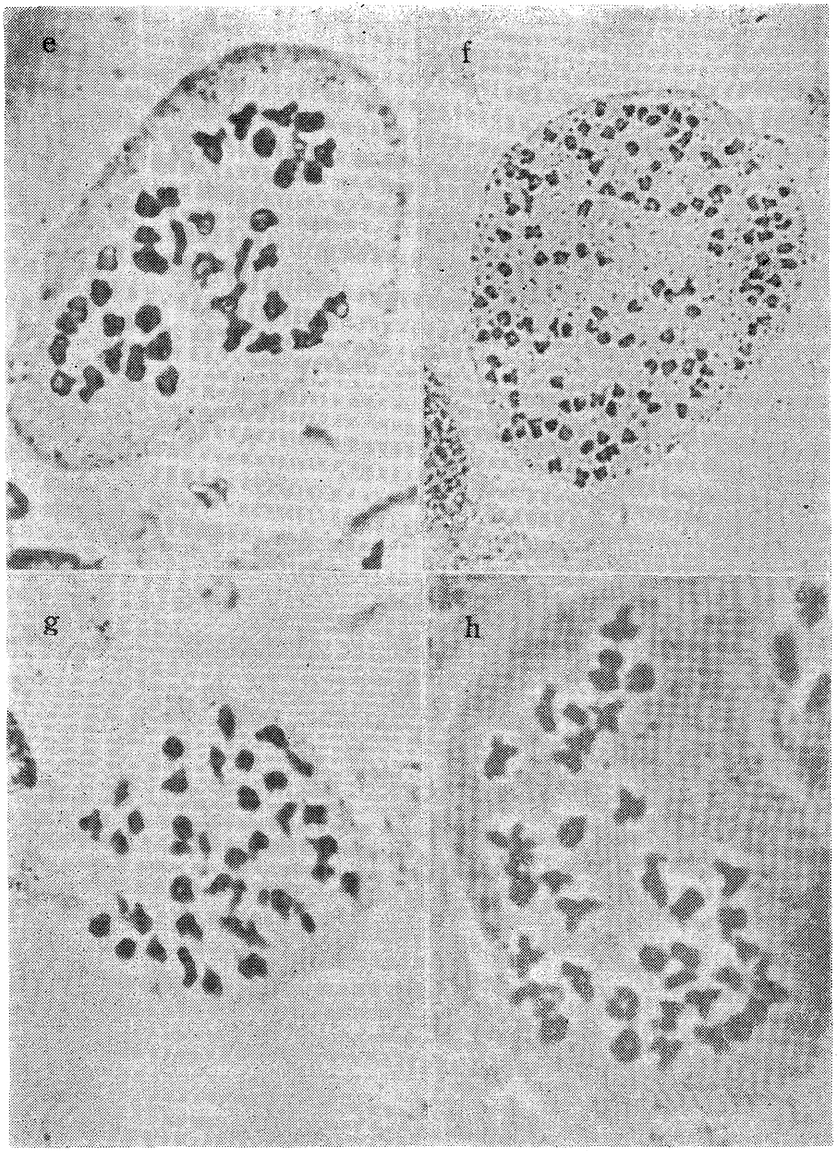

Fig. e. A spore mother cell of *Ctenitopsis sinii*, showing 41 bivalents. × c. 12000. Fig. f. A spore mother cell of *Diplazium doederleinii*, showing 123 bivalents. × c. 600. Fig. g. A spore mother cell of *D. pin-faense*, showing 41 bivalents. × c. 1000. Fig. h. A spore mother cell of *D. wichurae* var. *amabile*, showing 41 bivalents. × c. 1200.

some hesitation. The present material was obtained from Amakusa, Kumamoto Prefecture and has been cultivated at the Koishikawa Botanical Gerdens. The gametic chromosome number is 41 in many spore mother cells at the first meiotic metaphase (Fig. g). A sporangium contains 16 spore mother cells which give rise to 64 bilateral spores.

10) *Diplazium wichurae* (Mett.) Diels var. *amabile* Tagawa

Material was collected at Yakushima Island, Kagoshima Prefecture and has been cultivated at the Koishikawa Botanical Gardens. The gametic chromosome number is 41 at the first meiotic metaphase and diakinetic period (Fig. h). Each sporangium contains 16 spore mother cells and yields 64 bilateral spores. Previously the author found $n=41$ and $2n=82$ in the mother species, *D. wichurae*, in the materials from Shizuoka and Chiba Prefectures (Kurita, 1963). No cytological difference is found between this variety and the mother species.

11) *Dryopteris dickinsii* (Fr. et Sav.) C. Chr.

Material from Kiyosumi-Yama, Chiba Prefecture was examined cytologically. The haploid chromosome number is 82. The spore mother cells of this species give rise to active spores. The apogamous nature has been observed in this species by Momose (private communication). The number of spores in its sporangium, 32, also suggest apogamy.

12) *Dryopteris kominatoensis* Tagawa

The gross morphology of this species shows an interesting features, intermediate of *D. tokyoensis* and *D. monticola*. It is distributed in the north-east region of Japan, rather rarely. The material for the present work has been cultivated at the Koishikawa Botanical Gardens, since collected in Mikawa, Aichi Prefecture. The specimen showed irregularity at meiotic division, for there were many univalents and some bivalent chromosomes. In a spore mother cell, 80 univalents are accompanied by only one bivalent chromosome (Fig. i). Thus the total chromosome number is estimated to be 82, and the species appears to be diploid hybrid. Since *D. tokyoensis* from Mt. Bandai, Fukushima Prefecture (Kurita, 1961) and *D. monticola* from Nikko, Tochigi Prefecture (described below) have 41 gametic chromosomes is a spore mother cell, it seems quite probable that the present species is their hybridized derivative.

13) *Dryopteris monticola* (Makino) C. Chr.

Material was collected at Nikko, Tochigi Prefecture. In the first meiotic division 41 bivalent chromosomes are counted. Sporogenesis is regular and perfect, 16 spore mother cells in each sporangium give rise to 64 bilateral spores. This species is thus considered a sexually reproducing diploid.

14) *Dryopteris toyamae* Tagawa

This species is very rare. It much resembles *D. sieboldii* which has 82 chromosomes in haploid (Kurita, 1962), and some taxonomist consider it a variety of the latter. However, it is distinguishable from the latter, for it has bipinnatified fronds. The material was obtained at the Koishikawa Botanical Gardens. Originally it was collected at Kurobari-Yama Kumamoto Prefecture. Cytological study revealed an aberrant sporogenesis in this species. Both univalents and bivalents appear at the first meiotic division, and the spore mother cells never give rise to normal tetrads as we see in the hybrid. About 30 bivalents and 60 univalents are observed in a single spore mother cell. The somatic chromosome number of this species is estimated to be 123 which is three times the basic chromosome number, 41, of the genus *Dryopteris*. Thus the author considers that this species might be a triploid hybrid between the tetraploid *D. sieboldii* and an unknown diploid, or a hybrid between an unknown tetraploid species and a diploid race of *D. sieboldii*, which however could not be found yet.

15) *Hemionitis arifolia* Moore

H. arifolia is not found in Japan. The material used is of Formosan origin that has been cultivated at the Koishikawa Botanical Gardens. At the late diakinetic stage, 120 bivalent chromosomes are distinct in many spore mother cells (Fig. j.). Manton and Sledge (1954) already reported the same number in the specimen from Ceylon. They confirmed the apogamy in this species, since root tip cells and spore mother cells at meiosis showed 120 chromosomes respectively. Further they confirmed it in prothallia raised from wild spores. The present material shows 8 spore mother cells in a sporangium which eventually will contain 32 tetrahedral spores; it is an apogamous tetraploid (x=30) species like the one from Ceylon.

16) *Hypodematium fauriei* (Kodama) Tagawa

Material was collected at Sano, Tochigi Prefecture. At the first meiotic metaphase and at late diakinesis, 80 bivalent chromosomes are counted (Fig. k). Since Mehra and Loyal (1956) counted n=41 in the Himalayan species, *H. crenatum*, it appears that there are two different basic numbers, 41 and 40, in this genus. The present material is a tetraploid based on 40. Copeland (1917) placed *Hypodematium* near to *Woodsia*. Tagawa (1959) synthesized the opinions of

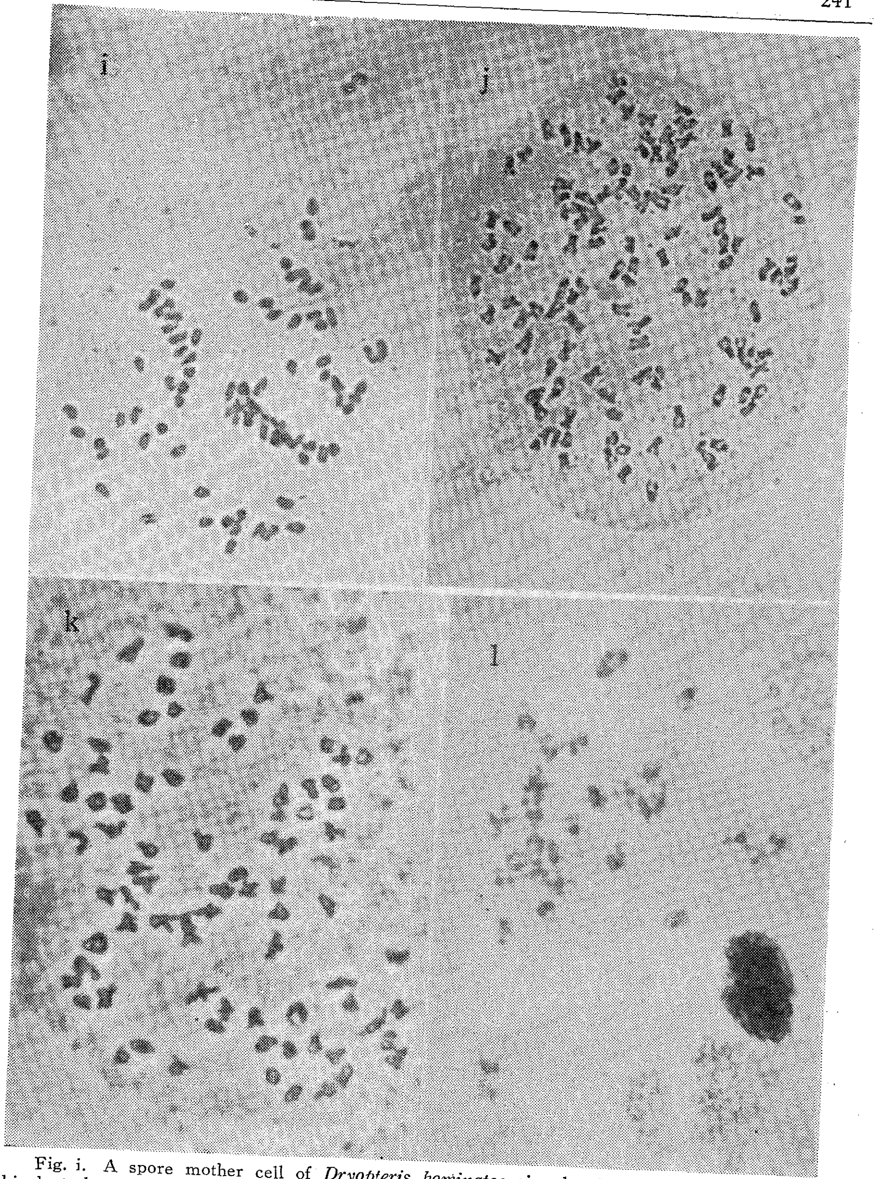

Fig. i. A spore mother cell of *Dryopteris kominatoensis*, showing 80 univalents and one bivalent chromosome. ×. c. 600. Fig. j. A spore mother cell of *Hemionitis arifolia*, showing 120 bivalent chromosomes. × c. 600. Fig. k. A spore mother cell of *Hypodematium fauriei*, showing 80 bivalents. × c. 800. Fig. l. A spore mother cell of *Struthiopteris nipponica*, showing 31 bivalent chromosomes. ×. c. 800.

Holttum and Alston and divided Aspidiaceae (sensu Copeland) into four families; Aspidiaceae (sensu stricto), Lomariopsidaceae and Athyriaceae. He included the present genus in his Aspidiaceae. On the other hand, Ching (1940) considered it as a genus of his Thelypteridaceae. If the setose hair in *Hypodematium* is seriously taken into account of, this genus should be considered thelypteroid. But inreality the hair in this genus is not so constant as in other members. Iwatsuki (1963 and 1964) declared that this genus, despite of the hair, does not belong to thelypteroid series, but may belong to athyrioid series, judging from many other distinctive characters. Cytological data agree with Iwatsuki, for the basic chromosome numbers 40 and 41 are not found in the thelypteroid ferns and Aspidiaceae (sensu stricto) always show $x=41$ but never $x=40$, whereas $x=40$ and $x=41$ are very common among the athyrioids.

17) *Polystichum gemmiferum* Tagawa

This species is rather rare, found in Yakushima Island, Kagoshima Prefecture. It produces gemma on the rachis. It has been cultivated at the Koishikawa Botanical Gardens where the material for study was obtained. The number of gametic chromosomes is 41. Sporogenesis is normal, producing 64 bilateral spores from 16 spore mother cells. Thus it must be a diploid species which propagate sexually.

18) *Struthiopteris amabilis* (Makino) Ching

This is an endemic species of Japan, fairly common in rocky regions. Specimens were collected at Ogasa-Yama, Shizuoka Prefecture and at Kiyosumi-Yama, Chiba Prefecture. Meiosis shows 31 bivalent chromosomes at the first division. Each sporangium, starting with 16 spore mother cells, yields 64 bilateral spore.

19) *Struthiopteris niponica* (Kunze) Nakai

This is also a Japanese endemic species very common in hilly districts, sometimes reaching a high level of about 2000 m. in central Japan. Specimens were collected at Kikugawa, Shizuoka Prefecture. The gametic chromosome number is 31 (Fig. 1). The 16-celled sporangia produce 64 bilateral spores. Previously the author (1960) examined the same species from Mt. Kumotori at the high of about 1900 m. above the sea level, and counted approximately 29 gametic chromosomes, but the preparation then used was not in a good condition, so that a re-examination of the mountain-born specimens is needed.

The gametic chromosome number 31, as found presently, has not been known before in the family Blechnaceae, though it could be foreseen by the finding of $2n=124$ in *B. occidentale* (Abraham, Ninan and Mathew, 1962). Including this new figure the family now has five basic chromosome numbers, 28, 31, 32, 33, 34 and 35.

The systematic position of Blechnaceae in particular will be discussed in a late paper.

Summary

1) Gametic chromosome numbers were counted in 19 fern species, *Adiantum edgeworthii* ($n=30$), *A. capillus-veneris* ($n=30$), *A. monochlamys* ($n=60$), *Athyrium pinetorum* ($n=40$), *Bolbitis subcordata* ($n=41$), *Camptosorus sibiricus* ($n=36$), *Ctenitopsis sinii* ($=41$), *Diplazium doederleinii* ($n=123$), *D. wichurae* var. *amabile* ($n=41$), *D. pin-faense* ($n=41$), *Dryopteris dickinsii* ($n=82$), *D. kominatoensis* ($2n=82$, irregular meiosis), *D. monticola* ($n=41$), *D. toyamae* ($2n=$c. 123, irregular meiosis), *Hemionitis arifolia* ($n=120$), *Hypodematium fauriei* ($n=80$), *Polystichum gemmiferum* ($n=41$), *Struthiopteris amabilis* ($n=31$) and *S. niponica* ($n=31$).

2) *Dryopteris kominatoensis* was considered a hybrid of *D. tokyoensis* and *D. monticola.*

3) The finding of $n=80$ in *Hypodematium fauriei* suggested a close relation of the genus *Hypodematium* to athyrioid, rather than to dryopteroid or thelypteroid ferns.

4) *Ctenitopsis sinii* was considered to be more related to *Ctenitis* than *to Tectaria.*

5) A new basic chromosome number, 31, was found in the family Blechnaceae.

The author wishes to express his cordial thanks to Dr. S. Momose, Prof. of Chiba University and to Dr. M. Nishida, Assist. Prof. of Chiba University, for their kind guidance given during the course of the work.

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19種のシダ類の染色体数を報告する。観察の結果，ホウライシダとオトメクジャクがn=30，ハコネシダがn=60，イヌアミシダがn=120，サツマシダ，ヘツカシダ，ミヤマベニシダ，ヒメノコギリシダ，フクレギシダ，コモチイノデがそれぞれn=41，シマシロヤマシダがn=123，オオクジャクがn=82，クモノスシダがn=36，タカネサトメシダがn=40，キンモウワラビがn=80，オサシダとシシガシラがn=31，ナガサキシダモドキが2n=c. 123，タニヘゴモドキが2n=82であることが知られた。

ナガサキシダモドキは羽状に浅〜中裂する点で母種のナガサキシダと区別される興味深いものであるが，減数分裂時において多数の1価染色体と少数の2価染色体が見られ，その体細胞における染色体数は約123であると考えられる。したがってナガサキシダ(n=82)と未知の種の交雑したものか，あるいは2倍体(n=41)のナガサキシダの一系と4倍体の未知の種とが交雑したものと考えることが出来る。

タニヘゴモドキは形態的にタニヘゴとミヤマベニシダの中間形を示すものであることは良く知られている事実であるが，細胞学的にみても両種の交雑によって生じたものであることが支持出来る。

キンモウワラビの分類学的位置については多くの人々の論議があるが，染色体数の面から考えると，岩槻氏の論ずるようにメシダ群に近縁のものと考えることが出来る。

シシガシラ科には，今日までに28，32，33，34，35の基本染色体数が知られていたが，筆者は新たに31の基本数の存在することを確認することが出来た。シシガシラ科の系統的な位置に関しては次報で詳細に論ずるつもりである。